DETERMINATION OF FLUBENDAZOLE AND METABOLITES IN EGG S AND POULTRY MEAT WITH LC-MS/MS

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1. INTRODUCTION

Flubendazole (FLUB) is a broad-spectrum benzimidazole anthelmintic, effective against endoparasites such as gastro-intestinal roundworms, gapeworms and tapeworms (2). This drug is widely used in the veterinary medicine of poultry. An administration of flubendazole can result in the presence of residues of the parent compound FLUB and its hydrolysed metabolite (HMET) and/or its reduced metabolite (RMET) in eggs and meat. Some chromatographic methods have been published to determine flubendazole in eggs and/or muscle (1, 8, 9, 11). Only a few residue depletion data in eggs of laying hens are available in the literature (1, 8). By EEC Council Regulation No 2377/90 and updates (3), the EU sets the MRLs for FLUB in eggs at 400 µg kg⁻¹ and for FLUB + HMET in poultry muscle at 50 µg kg⁻¹. This poster shows the optimization, the validation and the application of a quantitative and sensitive LC-MS/MS analytical method for the determination of FLUB, HMET and RMET in eggs and poultry muscle. In this study, the excretion of flubendazole and its metabolites in turkey muscle and liver after oral administration of flubendazole at two concentration levels was examined.

2. MATERIALS AND METHODS

Because of good results in former research work on benzimidazole drugs in milk (4), the benzimidazole analytes were extracted with ethyl acetate after the sample mixture had been made alkaline (7). The HPLC separation was performed on a reversed phase C₁₈ column using a mobile phase consisting of 0.04 M ammonium acetate (1, 8, 10) adjusted to pH 5.2 (A) and acetonitrile (B). Gradient elution was applied and the programme consisted of 50A:50B (0 min), 50A:50B to 25A:75B (0-3 min), 25A:75B (4-5 min), 25A:75B to 50A:50B (6-7 min) and 50A:50B (8-15 min). The flow rate was 0.25 mL min⁻¹ and the injection volume was 10 µL. The analytes were detected and identified with a tandem quadrupole mass spectrometer. Atmospheric pressure electro spray ionization in the positive mode (ESI⁺) was applied. FLUB, HMET, RMET and the IS were determined with MS/MS by the multiple reaction monitoring function of the transition of the molecular, parent ion to the most abundant daughter ion.

The method is completely validated conform the EU criteria of decision 93/256/EC (6) for determination of drug residues. The validation parameters are linearity of response, matrix calibration curve, extraction recovery, limit of detection (LOD) and limit of quantification (LOQ), trueness, repeatability and specificity.

The discussed method was applied to a pharmacokinetic study with turkeys (5). In two pens, the turkeys were fed medicated feed containing 19.9 and 29.6 mg kg⁻¹ flubendazole (Flubenol 5%, Janssen-Cilag, Beerse, Belgium) (State Analysis Laboratory, Tervuren, Belgium) for seven consecutive days during week 13 and week 15 of age for females and males respectively. Three male and three female turkeys were weighed and slaughtered at different ages according to the flubendazole feeding schedule, just before the start, daily during the administration and 2, 4 and 6h, 1, 2, 5 and 7 days post administration. At each time the same muscle group of breast and thigh and the liver were removed, frozen and stored at −18°C until investigation.

3. RESULTS AND DISCUSSION

The proposed MS detection method operating in the MS/MS mode is very selective and very sensitive. The limits of detection are around or lower than 1 µg kg⁻¹. A representative view of the separation and the detection
of FLUB, the metabolites and the IS of a blank egg sample spiked at 10 µg kg\(^{-1}\) is shown in the chromatogram in figure 1.

![Chromatogram of a spiked egg sample at 10 µg kg\(^{-1}\) with a mixture of FLUB, HMET, RMET and IS](image)

The validation parameters were completely in accordance with the criteria of the European rules (6). The over-all extraction recovery values for FLUB, HMET and RMET in eggs (fortification levels 200, 400 and 800 µg kg\(^{-1}\)) and muscle (fortification levels 25, 50 and 100 µg kg\(^{-1}\)) were respectively 77, 78, 80 and 92, 95 and 90%. The trueness (fortification levels 400 and 50 µg kg\(^{-1}\) respectively for eggs and muscle), expressed as percentage of the added values for these analytes were respectively 89, 100, 86 and 110, 110 and 98%. The LC-MS/MS confirmatory method operating in the MS/MS mode is very sensitive. The LODs for FLUB, HMET and RMET in egg and muscle were respectively 0.19, 0.29, 1.14 and 0.14, 0.75 and 0.31 µg kg\(^{-1}\). The LOQs were respectively 1, 1, 2 and 1, 1 and 1 µg kg\(^{-1}\).

One day after the end of the animal treatment, the mean sum of the FLUB + HMET residue values in thigh and breast muscle declined to around or below the MRL (50 µg kg\(^{-1}\)) and were respectively 36.6 and 54.1 µg kg\(^{-1}\). The corresponding values with the higher dose of 29.6 mg kg\(^{-1}\) were respectively 101.7 and 119.7 µg kg\(^{-1}\) (5). Figure 2 represents the mean FLUB and HMET residue values in breast and thigh muscle during and after the administration of flubendazole medicated feed at the recommended dose of 19.9 mg kg\(^{-1}\).

4. ACKNOWLEDGEMENTS

The authors wish to thank Janssen Animal Health (Beerse, Belgium) for the financial support of the animal tests. The donation of the analytical standards and the veterinary products is also very much appreciated. The authors also thank K. Haustrate of the State Analysis Laboratory (Tervuren, Belgium) for the analysis of the feed samples.
Figure 2. FLUB and HMET residues in turkey breast and thigh muscle during and after oral administration of 19.9 mg flubendazole kg\(^{-1}\) of feed

5. REFERENCES